REMARKS

1. Introduction

The presently outstanding Non-Final Office Action contains rejections of all claims but claim 20. Claims 2, 4, 7, 13 and 20 remain pending in the case. Heretofore, Applicants received an indication of allowability of claim 13 insofar as it recited, regarding expression of a heterologous antigen,

"an antigen of *Vibrio cholera*, an antigen of a microorganism which causes a respiratory infection, an antigen of a microorganism which causes a sexually transmitted disease, an hepatitis B surface antigen."

All remaining claims were therefore previously (by Amendment dated 8/30/2004) made to depend, directly or indirectly, from a single independent claim 13, which was amended to include

"an antigen of a pathogenic virus, an antigen of a pathogenic bacteria, and an antigen of a microorganism which causes an enteric infection."

The amendments previously made to allowed claim 13 incorporated limitations from previously presented claims 8, 9 and 12, which had been previously rejected only over Collier et al. 5,451,519. As discussed below, Collier '519 teaches the cloning of endonuclease restriction genes. The terms "antigen" or antigenicity are nowhere in the reference.

Support for the amendment to claim 2 may be found, e.g., at paragraphs [00164] and [00232]. Support for the amendment to claims 13 and 20 may be found in Example 2A, Example 2B and Example 3. These examples show the protective efficacy of Dam derivatives.

Turning now to the presently outstanding Office Action, the Objections and Rejections of the Detailed Action of the 11/12/2004 Office Action are set forth in the same order as presented in that paper.

2. Claim Objections (Office Action Paragraph 3)

Claims 2, 4 and 7 were objected to because they depend from a later presented claim.

Response

MPEP 608.01(n) provides:

"During prosecution, the order of claims may change and be in conflict with the requirement that dependent claims refer to a preceding claim. Accordingly, the numbering of dependent claims and the numbers of preceding claims referred to in dependent claims should be carefully checked when claims are renumbered upon allowance."

In the interests of furthering prosecution in the subject case, Applicants, through their attorney, hereby offer to renumber claims 2, 4 and 7 upon allowance.

3. Rejection of Claims 13, 2, 7 and 4 under 35 USC §102(b) over Torreblanca et al. (Office Action Paragraphs 4-5)

Torreblanca et al. is said to disclose a composition that is immunogenic. It is further said to disclose

"a diluent (a species of the instantly claimed excipients), specifically nutrient broth with added NaCl or E-medium without citrate together with glucose or lactose."

The disclosed mutant further is said to comprise a heterologous nucleotide encoding "an antigen of a microorganism that causes an enteric infection," namely the *E. coli dam* gene. The altered Dam activity in the reference is caused by the presence of a second heterologous nucleotide sequence, namely an inserted transposon Tn10dTet.

Response

This rejection is respectfully traversed. The Torreblanca et al. reference does not

disclose all recited elements of the rejected claims. It does not disclose an immunogenic composition. It does not disclose a pharmaceutically acceptable excipient. It does not disclose an "attenuated form of live bacteria." It does not disclose a second heterologous immunoprotective antigen, as now recited in claim 13.

(a) Teachings of Torreblanca et al.

The teachings of Torreblanca et al. are summarized in the Abstract, which begins,

"Mutants of Salmonella typhimurium lacking DNA adenine methylase were isolated; they include insertion and deletion alleles... Dam- mutants of S. typhimurium resemble those of E. coli in the following phenotypes: (1) increased spontaneous mutations, (2) moderate SOS induction, (3) enhancement of duplication segration, (inviability of *dam* recA and *dam* recB mutants, and (5) suppression of the inviability of the *dam* RecA and *dam* RecB combinations by mutations that eliminate mismatch repair."

In the course of their studies, the authors used the "nonhomologus transduction procedure" to create *dam* locus insertions, and isolated *dam*-mutants. The strains were isolated and grown on solid agar, so that replica plating could be employed. Both insertion and deletion mutants were obtained. The deletion alleles were obtained by selecting the sensitive derivatives of Tn10dTet insertions in *dam*. If the sensitive derivatives were obtained on plates lacking an aromatic mix supplement, only AroB+ Tet sensitive derivatives are obtained ((P. 24, col. 1).

Torreblanca et al. also characterized the *dam*- mutants of *S. typhimurium* by adding back a Dam function, a technique referred to as "complementation." The authors found that introducing an *E. coli dam* gene-containing plasmid into *dam- S. typhimurium* restored wild type properties to the recipient strain. These colonies were grown on NB plates for selection (p. 16, para. 5) , and their colony morphology was examined on green (or blue) plates (Fig. 2 legend, p. 19).

(b) Differences in claims

Claim 13 recites "an immunoprotective composition" having live bacteria which is attenuated and further having a heterologous immunogenic antigen. There is no suggestion in Torreblanca et al. that an immunoprotective composition is prepared. There is no suggestion there of immunogenicity, or of a preparation that could even be characterized as to immunogenicity.

There is no suggestion in Torreblanca et al. that the live bacteria are attenuated. There is also no suggestion in Torreblanca et al. that the *E. coli dam* gene, the purported heterologous antigen, is immunogenic or immunoprotective.

The present specification teaches that Dam- vaccines elicit ectopic expression of multiple proteins that enhance the immunoprotective effect of the vaccines. The immunoprotection is stronger than that afforded by wild type *dam*+ organisms (See Example 2B, para. 00259). Furthermore, the immunoprotection elicited by Dam- phenotype bacteria cross protective against other strains; and affords protection 10^4 above the lethal dose required to kill comparable to immunization with wild type organisms (See Example 3, para. 00262). Furthermore, as disclosed in Example 3, the presently recited composition confers immunity after the attenuated bacteria have been cleared from the system. Previous attenuated vaccines have only shown cross protection during the time that the vaccine strain was present in the host tissues. See Heithoff et al. "Salmonella DNA Adenine Methylase Mutants Confer Cross Protective Immunity," Infection and Immunity 69(11): 6725-6730 (2001).

There is no suggestion in any of the references cited that the cultures that were used would have any of these properties.

With regard to claim 2, as presently amended, Torreblanca et al. use a

"'nonhomologous' transduction procedure," as referenced on page 16, second column. The transposons used may insert themselves randomly into various locations in the genome. A *dam* site may be disrupted (typically in a polar manner), or not, and other sites may be disrupted as well. Thus the authors do not "specifically" alter the *dam* activity. Their technique is also not a "genetically engineered change," as recited in claim 7.

4. Rejection of Claims 13 and 7 under 35 USC §102(e) over Kleanthous et al. 6,585,975 as evidenced by Torreblanca et al. (Office Action Paragraph 6)

Kleanthous et al. is said to disclose an immunogenic composition that comprises an excipient in combination with an inactivated *S. typhimurium dam* gene, and further having a first heterologous nucleotide sequence coding for a heterologous antigen.

Response

This rejection is respectfully traversed. **The reference relies on a priority document that lacks any disclosure relevant to the present claims.**

The present application related back to applications having priority dates of Feb. 2 1999 and May 5, 1999. Support for the present claims may be found in those documents. Applicants' Science paper, "An essential role for DNA methylation in bacterial virulence," *Science* 284:967 appeared 7 May 1999. The Kleanthous patent application was not filed until Nov. 1, 1999.

A review of the Kleanthous et al. priority document, PCT/US98/08890, filed 4/30/98, entitled "Anti Helicobacterr vaccine composition for use by the subdiaphragmatic system route, and combined mucosal/parental immunization method," shows that it does not disclose attenuated *S. typhimurium*, as cited by the Examiner. The PCT publication is directed to the manufacture of a pharmaceutical composition derived from *Helicobacter* and intended to treat a *Helicobacter* infection.

Furthermore, while the cited and later filed Kleanthous et al. U.S. 6,585,975 does disclose the use of *Salmonella* vectors for vaccination against *Helicobacter* infection, it does not enable the preparation of *dam- Salmonella* vectors.

5. Rejection of Claims 13, 2, and 7 under 35 USC §102(b) over Bandyopadhyay et al. (Office Action Paragraph7)

Bandyopadhyay et al. was cited as disclosing a composition that is immunogenic and comprises "a diluent (a species of the instantly claimed excipients), specifically minimal media containing 1% casamino acids."

Response

This rejection is respectfully traversed. Bandyopadhyay et al. disclose the cloning of the *Vibrio cholerae dam* gene. **There is no disclosure or suggestion of preparing an immunogenic or immunoprotective composition, nor is there any suggestion that the transformed strains were attenuated.**

To equate a bacteria grown in culture with the recited "immunogenic composition" having a "pharmaceutically acceptable excipient" is to simply ignore recited claim limitations "pharmaceutically acceptable."

The specification does NOT define "pharmaceutically acceptable excipient" to include any diluent. This is particularly true where the diluents in question are in fact bacterial growth media. One of ordinary skill in the art would not expect a bacterial culture to be intended for administration to a living organism. The specification refers to a pharmaceutically acceptable excipient as follows:

[0167] Preferably, the compositions comprise a pharmaceutically acceptable excipient. A pharmaceutically acceptable excipient is a relatively inert substance that facilitates administration of a

pharmacologically effective substance. For example, an excipient can give form or consistency to the vaccine composition, or act as a diluent. Suitable excipients include but are not limited to stabilizing agents, wetting and emulsifying agents, salts for varying osmolarity, encapsulating agents, buffers, and skin penetration enhancers. Examples of pharmaceutically acceptable excipients are described in Remington's Pharmaceutical Sciences (Alfonso R. Gennaro, ed., 19th edition, 1995).

This does not mean that any diluent would be a pharmaceutically acceptable excipient.

Bandyopadhyay et al. disclose the cloning and characterization of the *dam* gene of *Vibrio cholerae.* As part of their studies, the authors transformed known *E. coli* dam- strain GW3810 (isolated in prior work by others from transposon mutagenesis). The cloned *Vibrio cholerae dam* gene, contained on plasmids introduced into the *E. coli* strain, was found to complement the missing *dam* gene product. Fig. 3 legend describes the preparation of the ^{35}S labeled *V. cholerae* Dam protein. The *E. coli* host cells containing the *V. cholerae* plasmid were grown in minimal media, irradiated with uv light (to induce Dam expression), and further incubated. Then, 200 μg of D-cycloserine was added. D-cycloserine is an antibiotic which inhibits cell wall synthesis. The cells were then harvested, suspended in fresh sulfur-depleted medium, incubated and the proteins were labeled with ^{35}S labeled methionine. The cells were then harvested, washed and lysed for gel analysis.

Bandyopadhyay give no suggestion of, or reason for preparing a therapeutic composition, as evidenced by the use of toxic antibiotics and radioactive materials in their method. The instantly claimed "pharmaceutically acceptable excipients" are simply not present, suggested, or workable with the procedures and preparations described in this reference. The specification does not define pharmaceutically acceptable excipients to include any diluent. It simply states, as is common knowledge, that certain acceptable excipients may act as diluents. See paragraph [00152], "For example, an excipients can give form or consistency to the vaccine composition, or act as a diluent."

6. Rejection of Claims 13, 2, and 7 under 35 USC §102(b) over Collier et al. U.S. 5,451,519 (Office Action Paragraph 8)

Rejection

The non-Final Office Action states that Collier et al. discloses a composition that is immunogenic. It is further said to disclose

"a diluent (a species of the instantly claimed excipients, definition provided in the instant Specification), specifically liquid media in 2YT broth (See Col. 20, line 5; col. 20, lines 55-17). The instant Specification defines excipient to include diluents."

It is further stated that the *E. coli dam* mutant disclosed has a heterologous nucleotide sequence coding for *E. coli* antigens EcoR1 endonuclease or Eco R1 methyltransferase or B-galactosidase from a different strain of *E. coli*.

Response

This rejection is respectfully traversed. Collier et al. '519 does not disclose all recited elements of the rejected claims. It does not disclose an immunogenic composition. It does not disclose a pharmaceutically acceptable excipient; and it does not disclose an "attenuated form of live bacteria," as recited in claim 13.

(a) Teachings of Collier et al.

Collier et al disclose, "a method for cloning genes that encode restriction endonucleases by altering the level of a methyl donor co-factor of a DNA methyltransferase that protects the DNA of a host cell from damage by a restriction endonuclease." (Abstract). In Example 3, the construction of a *dam- E. coli* strain that expresses beta

galactosidase in response to DNA damage is described. A *dam*- derivative of a well known *E. coli* MC 1061 was used. The construct contained a dinD promoter controlling the expression of beta galactosidase, so that beta galactosidase was expresses in response to SOS-inducible DNA damage by a restriction endonuclease. In Examples 3 and 4, cited in the Office Action, the strains were grown in 2YT broth containing the DNA damaging agent mitomycin D (Example 3) or containing kanamycin, ampicillin, chloramphenicol and chlortetracycline (Example 4).

(b) Differences in claim recitations

The "components" of Collier et al. are simply the growth media used for the various constructs, such as 2YT broth. Bacterial growth media is not a "pharmaceutically acceptable excipient" as recited in claim 13.

Furthermore, claim 13 recites certain heterologous antigens not taught or suggested by Collier et al. There is no discussion in this reference of specific antigens that may be incorporated into the recited composition. Thus, it is submitted that claim 13, as currently amended, and those claims dependent thereon are presently allowable.

Conclusion

It is believed that the present Amendments place the application in condition for allowance. Allowance of claims 2, 4, 7, 13 and 20 is respectfully requested. Such action, as well as the timely issuance of a Notice of Allowance is earnestly solicited. If a telephone

conference would be useful in this case, the Examiner is encouraged to call the undersigned at the number below.

Respectfully submitted,

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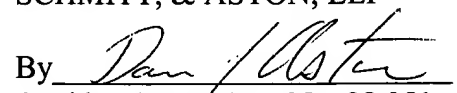
By 

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